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SCREENING FOR ANTIFUNGAL ACTIVITIES OF PROBIOTICS IN COMMON FERMENTED FOOD AGAINST *FUSARIUM SP.* AND *CLADOSPORIUM SP.*

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Screening for Antifungal Activities of Probiotics in Common Fermented Food Against *Fusarium sp.* and *Cladosporium sp.*

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This project report is submitted in partial fulfillment of the requirements for the Degree of the Bachelor of Science with Honours

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Screening for Antifungal Activities of Probiotics in Common Fermented Food Against *Fusarium sp.* and *Cladioporium sp.*

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ABSTRACT

Local production cannot supply fresh crops stocks in market. This is due to post-harvested crop that cannot maintain their freshness in long period of time because of contamination during post-harvested management. In addition, fungi can easily contaminated from poor storage handling. Thus, this study alternatively focus on fermented food which might act to slow or inhibit the growth of fungal pathogen such as *cultured drinks, budu,* and *cencalok.* Based on plug assay techniques, cultured drinks showed inhibitory effect against all the tested fungal (*Fusarium* white, *Fusarium* pink and *Cladosporium sp.*). Furthermore, *cencalok* showed inhibitory effect when tested to *Cladosporium sp.* It was revealed that cultured drinks which consists of specific lactic acid bacteria strains conferred highest antifungal activity.

Keywords: *antifungal activities, fermented food, Fusarium sp., Claiosporium sp.*

ABSTRAK

Penghasilan tempatan tidak dapat membekalkan hasil tanaman yang segar ke pasaran. Hal ini disebabkan oleh hasil tanaman yang dituai tidak dapat bertahan kesegarannya dalam jangka masa yang panjang kerana terdedah semasa pengurusan hasil tanaman. Tambahan pula, kulat mudah tercemar daripada pengendalian hasil tanaman yang lemah. Oleh itu, kajian ini secara alternatif memberi tumpuan kepada makanan yang telah difermentasikan seperti minuman kultur, budu dan cencalok. Berdasarkan teknik plug, minuman berkultur menunjukkan kesan anti-kulat terhadap semua fungi yang diuji (Fusarium white, Fusarium pink and Cladosporium sp.). Selanjutnya, cencalok menunjukkan kesan anti-kulat apabila diuji pada Cladosporium sp. Ia menunjukkan bahawa minuman berkultur yang mempunyai bakteria laktat asid strain yang spesifik mempunyai antikulat aktiviti paling tinggi.

Kata kunci: *aktiviti antikulat, makanan fermentasi, Fusarium sp., Cladosporium sp.*

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List of Abbreviations

AFS	Antifungal Substances
L.	*Lactobacillus*
LAB	Lactic Acid Bacteria
PDA	Potato Dextrose Agar
sp.	*Species*

CHAPTER 1: INTRODUCTION

Risen of retails stores of fresh fruit and vegetables are continue demanding especially in major urban cities (Man *et al.*, 2009). However, local producer unable to supply fresh vegetables and fruits in hypermarkets due to poor practices (Man *et al.*, 2009). Poor management such as improper handling, packaging and transporting of post-harvested crops lead to activation of microorganism due to physiological changes (Wilson *et al.*, 1991; Tournas, 2005).

Mould and yeast are most frequents spoilage microorganism of vegetables and food producing low quality and quantity of fresh products. Fungi which usually cause damage of fresh vegetables are *Botrytis cinerea*, various species of genera *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Pomopsis, Fusarium, Penicillium, Phytophthora, Pythium* and *Rhizopus spp.* (Coates & Johnson, 1997; Tournas, 2005). In meantime, plant diseases associated to plant pathogen causes high loss to crop productions and some species of fungi could produce toxin metabolites constituting potential health problems to human. Human health risks are commonly associated with direct consumption of food products contaminated with mycotoxin (Tournas, 2005).

Alternatively, fermented foods and beverages consist of lactic acid bacteria (LAB) believe to carry numerous nutritional and therapeutic properties which recently studied as food preservatives, antibacterial and antifungal agent. LAB are able to produce specific proteinaceous substances, bacteriocin which act as biopreservatives, enhances the shelf life of the food and capable to inhibit the growth of food pathogens included *Listeria, Clostridium, Staphylococcus, Bacillus ssp.* and *Enterococcus spp.* (Soomro *et* *al*., 2002). As well as in agricultural field, LAB are able to control fungal pathogen on post-harvested crop also attract some plant pathologist to carried out solution for plant disease. Shelf-life of fresh vegetables can be kept in longer duration with the potential of LAB, *Lactobacillus plantarum* (Sathe *et al*., 2007). LAB from different environment and from different genera and species can exhibit antifungal activity against number of common mould and yeast (Magnusson *et al*., 2003). Research on spectrum of antifungal activity of LAB against some species of mold genera, *Aspergillus, Penicillium* and *Fusarium* representing plant pathogen showed inhibition of the fungi especially on *Fusarium sp.* (Hassan & Bullerman, 2008). Thus, the proposed of this study to screening antifungal activity of fermented foods against selected fungi.

1.1 Objectives

- To determine antifungal activities of fermented foods by using plug assay method.

CHAPTER 2: LITERATURE REVIEW

2.1 Plant Pathogen

Vegetables are expected to contain relatively high numbers of microorganisms at harvest because of their contact with the soil during growth (Tournas, 2005). Generally, there are several microorganisms capable of proliferating on vegetables species of microorganisms can break through the protective outer layer of plant possess, grow and cause spoilage of crop production while the others microorganisms can only enter through wounds of broken plant tissue, grow and cause damage to the plant. Fungi which usually cause damage of fresh vegetables are *Botrytis cinerea*, various species of genera *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Pomopsis, Fusarium, Penicillium, Phytophthora, Pythium* and *Rhizopus spp.* (Coates & Johnson, 1997; Tournas, 2005).

2.1.1 *Fusarium sp.*

Fusarium is a filamentous fungus that commonly distributed in crops at tropical and subtropical areas (Dhoro, 2010). Most species of *Fusarium* are relatively abundant in the soil microbial community. *Fusarium* strain frequently can cause huge devastating range of plant diseases. Diseases that have been diagnose from *Fusarium sp.* are crown and root rots, stalk rots, head and grain blights and wilt of vascular disease are the most frequent recognized by pathologist (Summerell *et al.*, 2003). *Fusarium sp.* could also invaded fresh fruits and vegetables during storage causing rots and spoilage (Bullerman, 2003). Some *Fusarium sp.* has pathogenic characteristics and can cause diseases to human and animal because it produces mycotoxin and mycotoxicoses since the both organism involves in the process of post-harvested plant and consume in diet (Nelson *et al.*, 1994; Bullerman, 2003).

2.1.2 *Cladosporium sp.*

Clasdosporium frequently found on living and dead of plant tissue and some of their species are pathogenic towards plant and parasitize other fungi. Ecological distribution of *Cladosporium sp.* occurs in wide variety of host, survive in senescent plant tissues, biotrophic and necrotrophic manner (Walker *et al.*, 2016). This genus has reported on attacking post-harvested fruit such as apples, showing symptoms such as leaf spot and food (Barkat *et al.*, 2016; Walker *et al.*, 2016). Some of *Cladosporium sp.* also human pathogenic and researches have been identified the causal agent which is from species of *cladophialophora* (Bensch *et al*, 2012).

2.2 Fermented Food and Beverages

Fermented foods are products that involve the action of microorganisms or enzymes which enhances biochemical changes and denoting modifications to the food (Campbell-Platt, 1994). In terms of production and consumption of fermented product, it can be divided into three major group which are dairy food products, beverages and cereals while others groups include are meat, fish, legumes, miscellaneous (mushroom and *ragi*), starch crops where major in Africa, fruit (pickles fruit) and vegetables (kimchi) (Campbell-Platt, 1994; Achi, 2005). Indigenous fermentation usually required long period of time with inconsistent product quality become factor that limit the industries (Sim *et al.*, 2015). However, a few traditional fermentations in Asia have been upgraded to high technology production system which are isolated microbial strains were applied as starter culture for controllable fermentation in biotechnology development (Achi, 2005; Sim *et al.*, 2015).

2.2.1 Fermented Milks

According to International Diary Federation (1988) cited by Oberman and Libudzisz, fermented milk is a product prepared from skimmed milk or not skimmed milk, with specific cultures which is the microflora kept alive until marketing to consumer without any pathogenic germs (Oberman & Libudzsiz, 1998). Culture milks are classified as fermented milks with mesophilic organism growth between 20 °C and 30°C (Shiby & Mishra, 2017). Yakults and Vitagen are commercial cultured drinks can be found in Malaysia. Both cultured drinks consist of specific bacteria strains. Vitagen contains billions of probiotics known as *Lactobacillus acidophilus* and *Lactobacillus casei* which help improve digestive processes

(Vitagen.com, 2017). However, according to Yakult.co.in, Yakults specifically contains over 6.5 billion beneficial bacteria *Lactobacillus casei strain Shirota* (LcS) with proven health benefits. In addition, cheese and yogurt and sourcream also classified as fermented milks (Kroger *et al.*, 1992).

2.2.2 Fermented Fish

Fermented fish also commonly known as fish sauce are well known in Southeast Asia countries. Moreover, fish sauce is called in different terms for various countries such as *budu* (Malaysia), *kecap ikan* or *bakasang* (Indonesia), *nampla* (Thailand), *shottsuru* (Japan), *aekjeot* in Korea and *yeesu* or *uy-lu* in China (Sim *et al.*, 2015; Irianto, 2017). This fermentation process mixing fish and salt thoroughly at ratio 1:3 requires 3-12 months in fermentation tanks at ambient temperature (Irianto, 2017). Specifically, in Malaysia *budu* was prepared by mixing raw anchovies and salts. *Budu* as indigenous fermented fish sauce present as viscous brown liquid with grey colloidal fish have been separated from indigestible fish bone. Additionally, fermentation of *budu* involved microbial environment. Thus, *Lactobacillus spp., Lactococcus spp.,* and *Pediococcus spp.* were present in distribution of microbial strains during fermentation while *Micrococcus luteus* and *Staphylococcus arlettae* become dominant strain that might encourage desirable technologies properties which assist in *budu* fermentation (Sim *et al.*, 2015).

2.2.3 Fermented shrimp

Fermented shrimps product can be have three major categorized into sauce, pastes and lacto-fermented product with various names by local people of each country such as *cencalok* in Malaysia which are closely related to Malaccan food (Hajeb & Jinab, 2012). Fermented shrimps also known as *balao-balao* in Philippines and *jaloo koongsom* in Thailand (Hajeb & Jinab, 2012). Fresh small-sized shrimp washed with seawater, salt and rice are main ingredients involved in this fermentation process for 20 – 30 days (Huda, 2012). The fermentation undergo in sealed container with additional red colouring agent or tomato sauce as an option. Genus *Acetes* is most regular raw materials to produce shrimps sauce, paste and fermented products (Hajeb &Jinab, 2012). The product frequently used in cooking as seasoning, consumed as side dishes or staples in daily diets. Nowadays, *cencalok* are commercially found in market and packaging in glass bottles.

2.3 Lactic Acid Bacteria

According to Carr and colleagues, mention that lactic acid bacteria (LAB) consist of two groups, homofermenters and heterofermenters based on end product of fermentation with various numbers of genera (Carr *et al.*, 2002). Homofermentative lactics include the genera *Streptococcus* and *Pediococcus* while, heterofermentative lactics consist of the genus *Leuconostoc* and *Lactobacillus*. Generally, lactic acid bacteria may be characterized as gram-positive bacteria with rods or cocci morphologies, non-sporforming and acid tolerant. Lactic acid bacteria have been proposed to have several genera including *Betabacterium, Thermoobacterium, Streptobacterium, Streptococcus, Betacoccus, Microbacterium, Tetracoccus* which have been changed and known as *Lactobacillus, Streptococcus,* *Lactococcus, Vagococcus, Leuconostoc, Pediococcus* and *Tetragenococcus* (De Vuyst & Vandamme, 1994; Stiles & Holzapfel, 1997; Carr *et al.*, 2002).

2.3.1 Antifungal Activity of LAB

Few reviews of lactic acid bacteria as antifungal agent have been reported as information collection (Batish *et al.*, 1997; Schnürer & Magnusson, 2005; Dalié *et al.*, 2010). Many studies of lactic acid bacteria isolated from various sources such as natural honey, sourdough bread culture and environments including plant material and animal) against fungi have been conducted and showed antifungal activities (Magnusson *et al.*, 2003; Hassan & Bullerman, 2008; Bulgasem *et al.*, 2016). Isolation of lactic acid bacteria from fermented product, sourdough bread culture and *kunu* (non-alcoholic beverages produced from grains) showed antifungal activity towards *Fusarium sp.* and *Aspergillus sp.* by measuring clear zone of inhibition (Hassan & Bullerman, 2008; Oloisakin *et al.*, 2017).

CHAPTER 3: METHODOLOGY

3.1 Isolation of Fungi

3.1.1 *Fusarium* White

Fusarium white is fungi culture collections obtain from Agriculture Research Centre (ARC) isolated from black pepper. The fungi were isolated from the stock culture and transferred onto PDA. The fungi were kept maintained until the antifungal test.

3.1.2 *Fusarium* Pink

Fusarium pink is fungi culture collections obtain from Agriculture Research Centre (ARC) isolated from banana. The fungi were isolated from the stock culture and transferred onto PDA. The fungi were kept maintained until the antifungal test.

3.1.3 *Cladosporium sp.*

Spore drop technique was used to isolate single spore of fungi from chilli. Symptom of fungal appeared on chilli was cut. Then, surface sterilization was conducted by sterilizing with one time of 1% of chlorox and three times of distilled water (dH_2O). The cut sample was taped on the top of plate containing water agar (WA). Then, the plate was incubated in the container filled with water at bottom part of the container. The container was left at room temperature. The plate will be observed daily after incubated. When the spore germinated, the spore will be subculture onto half strength PDA. After that, the sub-cultured plate will be incubated at room temperature. Dilution of the fungi was conducted. Few single spore was picked and subculture on potato dextrose agar (PDA). The morphology of fungi was observed. The genus was identified by observing the spore of the fungi under microscope. The fungi were maintained until the antifungal test.

3.2 Antifungal Test

3.2.1 Plug Assay Technique

Plug diameter of 7 mm contained isolated fungi from maintained culture was placed at the centre of PDA mixed with fermented food samples (5% Vitagen, 5% *Budu* and 5% *Cencalok*). The growth of the fungi was marked daily until 7 days. (Hussin *et al*, 2016).

3.3 Data Collection

Colony growth of the fungi was measured by using ruler according to line drawn at the bottom of petri dish. A, B, C and D were labeled represents each radius. The growth rate was estimated by dividing the total colony diameter growth over total number of days by the colony growth (Hussin *et al*, 2016). The calculation of colony growth and growth rate of fungi recorded and repeated for each plate by following formula below,

Colony growth for Day 1:

$$D1 = \frac{(A+B+C+D)}{4}$$

Growth Rate of fungi:

$$R = \frac{[(D2-D1)+(D3-D2)+(D4-D3)+(D5-D4)+(D6-D5)+(D7-D6)]}{(N-1)}$$

N: Total number of day

3.4 Statistical Analysis

The data were collected and the results were displayed as the rank mean values, Man-Whittney U test. The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 24.0 (SPSS Inc.).

CHAPTER 4: RESULTS & DISCUSSION

4.1 Isolation of Fungi

4.1.1 *Fusarium* white & *Fusarium* pink

Fusarium colonies on agar appear to be cottony owing to dense growth of white hyphae. In addition, *Fusarium sp.* also produces various colours ranging from white, pink, salmon-pink and carminered to purple (Bullerman, 2003). Figure 1 and 2 showed different colour of mycelia of *Fusarium* white and *Fusarium* pink respectively.

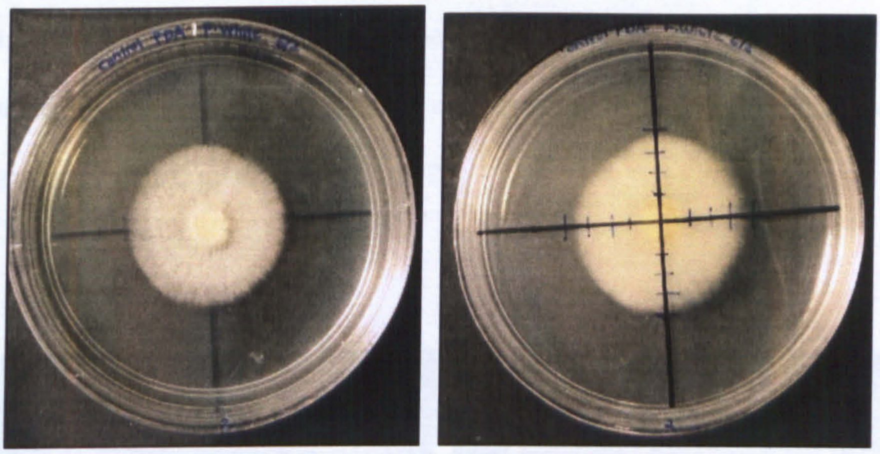

Figure 1. Morphology of *Fusarium* white. on PDA (Left:Top plate, Right:Bottom plate)

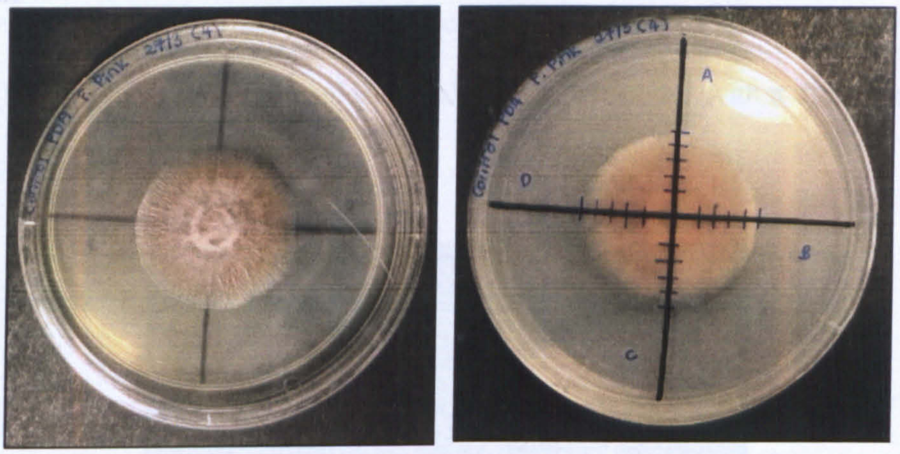

Figure 2. Morphology of *Fusarium* pink. on PDA (Left:Top plate, Right:Bottom plate)

4.1.2 *Cladoporium sp.*

Cladosporium has dark mycelia which may be brown to blakish-brown or gray-green colour. The bottom side of colony *Cladosporium* on agar produce very dark greenish-black or blue-black (Bullerman, 2003) (Figure 1 and Figure 2).

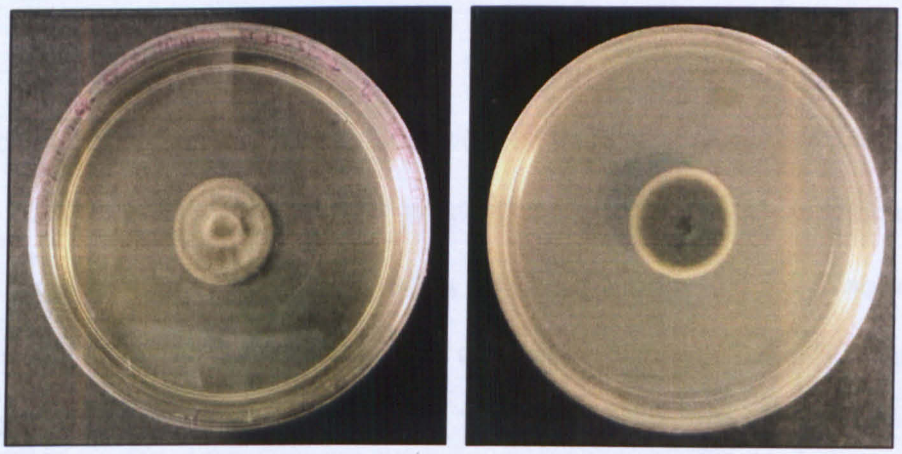

Figure 3. Morphology of *Cladosporium sp.* on PDA (Left:Top plate, Right:Bottom plate)

Cladosporium sp. produces many one celled-conidia, but two- and three celled forms are are common (Bullerman, 2003). The conidia noted to be single-celled with a distinct dark hilum (Figure 3).

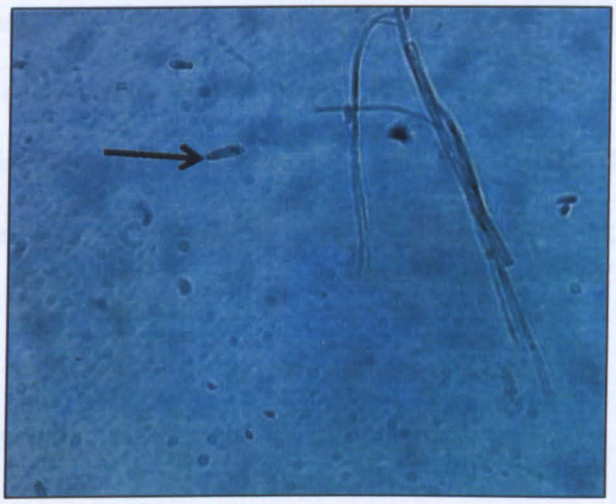

Figure 4. Spore morphology of *Cladosporium sp.*

4.2 Statistical Analysis

The data collected were analyzed by using SPSS software and the test used was non-parametric test, Mann-Whittney U test. This study test three fungi (*Fusarium* white, *Fusarium* pink and *Cladosporium sp.* on control potato dextrose agar (PDA) and three different fermented foods mix with PDA labeled as 5% Vitagen, 5% *Budu* and 5% *Cencalok*. All the fungi tested were associated as spoilage agent of fresh vegetables (Coates & Johnson, 1997; Tournas, 2005).

4.2.1 *Fusarium* white

The growth rate of *Fusarium* white on 5% Vitagen showed significantly difference compared to growth rate of *Fusarium* white on control PDA, $p<0.05$ (Table 1). Based on figure 5, the growth of *Fusarium* white on 5% Vitagen are slower than control PDA. The inhibition effect of 5% Vitagen might be caused by specific lactic acid bacteria (LAB), *Lactobacillus casei* and *Lactobacillus acidophilus* strains which consist in the culture drinks. The results also showed consistent inhibition effect of on two other fungi, *Fusarium* pink and *Cladosporium sp.* with significant, $p<0.05$ (Table 5 and Table 9) which could be considered as a good result. This study almost similar to Tropcheva *et al.* (2014), which few isolate LAB from fermented skimmed milk showed inhibitory effect against *Fusarium sp.*

Table 1. Comparison mean of growth rate of *Fusarium* white between Control PDA and 5% Vitagen

	Control PDA	5% Vitagen
Mean Growth	0.41	0.35
P Value	0.029	

Interestingly, the growth rate of *Fusarium* white on 5% *budu* and 5% *cencalok* had no significantly difference compared to growth rate on control PDA respectively, $p>0.05$ (Table 2 and Table 3). When compared to growth rate of *Fusarium* white, growth rate of *Fusarium* white on 5% *budu* and 5% *cencalok* are quite similar (Table 4). The content in different types of agar might affect the growth rate of the fungi. As mention by Dalié *et al.* (2010) in a review of LAB, the growth medium could impact the production of antifungal metabolites by LAB which includes low-molecular weight compound composed of organic acids, reuterin, hydrogen peroxide, proteinaceous compounds, hydroxyl fatty acids and phenolic compounds. In addition, Lindgren and Pleje (1983) stated that the antimicrobial activity of organic acids could be due to undissociated molecules. Figure 5 below showed comparison growth between 5% *budu* and 5% *cencalok* with control PDA. The grow of *Fusarium* white on both fermented foods, *budu* and *cencalok* observed faster than control PDA which show weak inhibitory effect of the fermented foods. This might be due to growth medium mixed with fermented food which could promote the growth of the fungi. However, there is no significant different when compared growth rate of *Fusarium* white on 5% *budu* and 5% *cencalok* respectively to control PDA.

Table 2. Comparison mean of growth rate of *Fusarium* white between Control PDA and 5% *Budu*

	Control PDA	5% *Budu*
Mean Growth	0.41	0.44
P Value	0.20	

Table 3. Comparison mean rank of growth rate of *Fusarium* white between Control PDA and 5% *Cencalok*.

	Control PDA	5% *Cencalok*
Mean Rank	0.41	0.48
P Value	0.057	

Table 4. Growth rate (cm/day) of *Fusarium* white on control PDA and three different of fermented foods

Growth rate of *Fusarium* white (cm/day)			
Control PDA	5% Vitagen	5% *Budu*	5% *Cencalok*
0.39	0.34	0.43	0.44
0.41	0.35	0.43	0.44
0.41	0.36	0.44	0.50
0.44	0.36	0.44	0.52

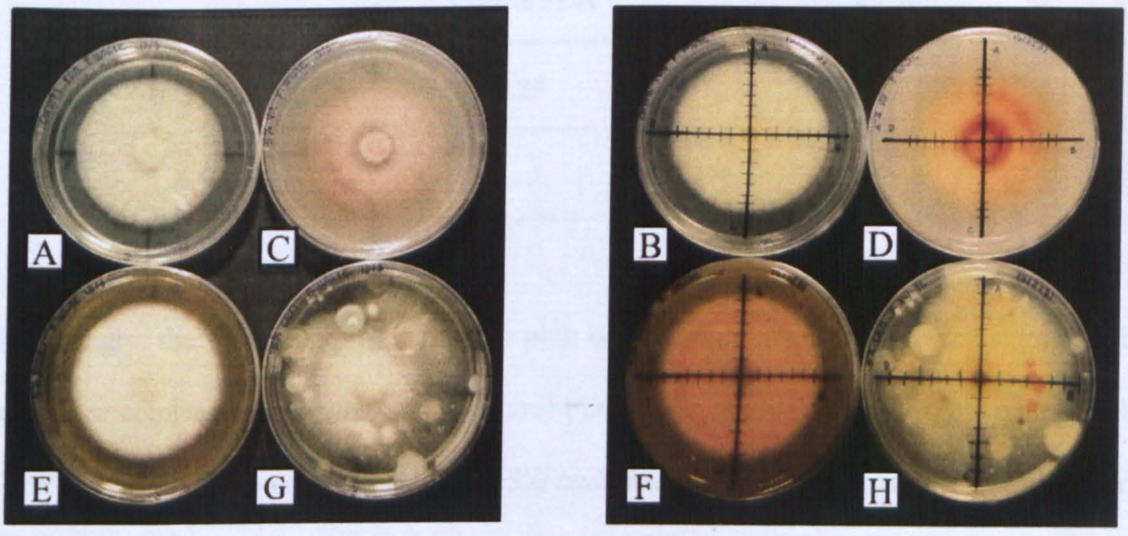

Figure 5. Growth of *Fusarium* white on PDA and 3 different fermented foods mixed with PDA. (a) top plate control PDA, (b) bottom plate control PDA (c) top plate 5% vitagen, (d) bottom plate 5% vitagen, (e) top plate 5% *budu*, (f) bottom plate 5% *budu*, (g) top plate 5% *cencalok*, and (h) bottom plate 5% *cencalok*.

4.2.2 *Fusarium* pink

The growth rate of *Fusarium* pink on 5% Vitagen showed significantly difference compared to growth rate of *Fusarium* pink on control PDA, $p<0.05$ (Table 5). Previous discussion had state that, this result might contribute from specific LAB that consists in the commercial cultured drinks, Vitagen. Culture drink, Vitagen might have stable ingredients of LAB as it resulted in consistent inhibitory effect against tested fungi. However, *budu* and *cencalok* still undergoes traditional fermentation process with no standard procedures even though the procedure are known based on previous study by Irianto (2017).

Table 5. Comparison mean of growth rate of *Fusarium* pink between Control PDA and 5% Vitagen

	Control PDA	5% Vitagen
Mean Growth	0.25	0.18
P Value	0.029	

Surprisingly, the growth rate of *Fusarium* pink on 5% *Budu* and 5% *Cencalok* significantly difference compared to growth rate on control PDA respectively ($p<0.05$) (Table 6 and Table 7). When compared to control PDA, both 5% *budu* and 5% *cencalok* showed weak inhibitory effect against *Fusarium* pink. The production of antimicrobial activity and substances of LAB might also have affected by pH values of medium (Batish *et al.*, 1997). Antifungal substances (AFS) was noted to have low level of production at lower pH while produce maximum AFS at pH 6.8 (Batish *et al.*, 1997). Moreover, when the agar has different pH, the texture of the agar might affect as there are different types of fermented food have been mixed with PDA which influences the growth of the fungi. Competition in microbial population between bacteria was observed in different pH (Lindgren & Pleje, 1983). Furthermore, the study also stated that lower water content reduced the growth rate of LAB due to low water activity.

Table 6. Comparison mean of growth rate of *Fusarium* pink between Control PDA and 5% *Budu.*

	Control PDA	5% *Budu*
Mean Growth	0.25	0.37
P Value	0.29	

Table 7. Comparison mean of growth rate of *Fusarium* pink between Control PDA and 5% *Cencalok.*

	Control PDA	5% *Cencalok*
Mean Growth	0.25	0.29
P Value	0.029	

In addition, table 8 and figure 6 showed that the growth rate of this fungi increase faster on 5% *budu* and 5% *cencalok* compared to control PDA. This might be due to factor abundant of nutrients that consist in both fermented foods which enhances the growth of the fungi (Dalié e al., 2010). PDA consists of potato infusion, dextrose, agar and distilled water. However, when *budu* and *cencalok* were mixed with PDA respectively, it might increase the nutrient contents of the agar which might promote the growth of the fungi. Protein sources from fish and shrimps that have been fermented could contribute enhancing the fungal growth.

Table 8. Growth rate (cm/day) of *Fusarium* pink on control PDA and three different of fermented foods.

Growth rate of *Fusarium* pink (cm/day)			
Control PDA	5% Vitagen	5% *Budu*	5% *Cencalok*
0.27	0.17	0.34	0.28
0.27	0.18	0.36	0.29
0.27	0.18	0.38	0.29
0.28	0.18	0.41	0.30

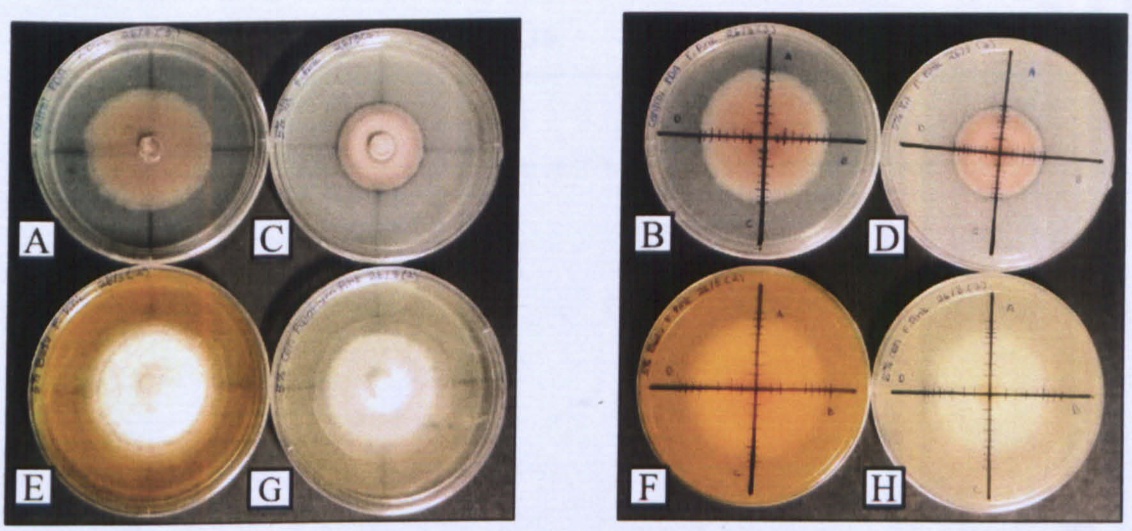

Figure 6. Growth of *Fusarium* pink on PDA and 3 different fermented foods mixed with PDA. (a) top plate control PDA, (b) bottom plate control PDA (c) top plate 5% vitagen, (d) bottom plate 5% vitagen, (e) top plate 5% *budu*, (f) bottom plate 5% *budu*, (g) top plate 5% *cencalok*, and (h) bottom plate 5% *cencalok*.

4.2.3 *Cladosporium sp.*

The growth rate of *Cladosporium sp.* on 5 % Vitagen showed significantly difference compared to growth rate of on control PDA, $p<0.05$ (Table 9). The rate growth of the Cladosporium sp. on 5% Vitagen agar is the slowest when compared to Cladosporium sp. growth on other mixed fermented food agar (Figure 7). Similar to previous discussions, specific strains LAB in the commercial cultured drinks might contribute the inhibitory effects growth of the fungi.

Table 9. Comparison mean of growth rate of *Cladosporium sp.* between Control PDA and 5% Vitagen.

	Control PDA	5% Vitagen
Mean Growth	0.16	0.048
P Value	0.029	

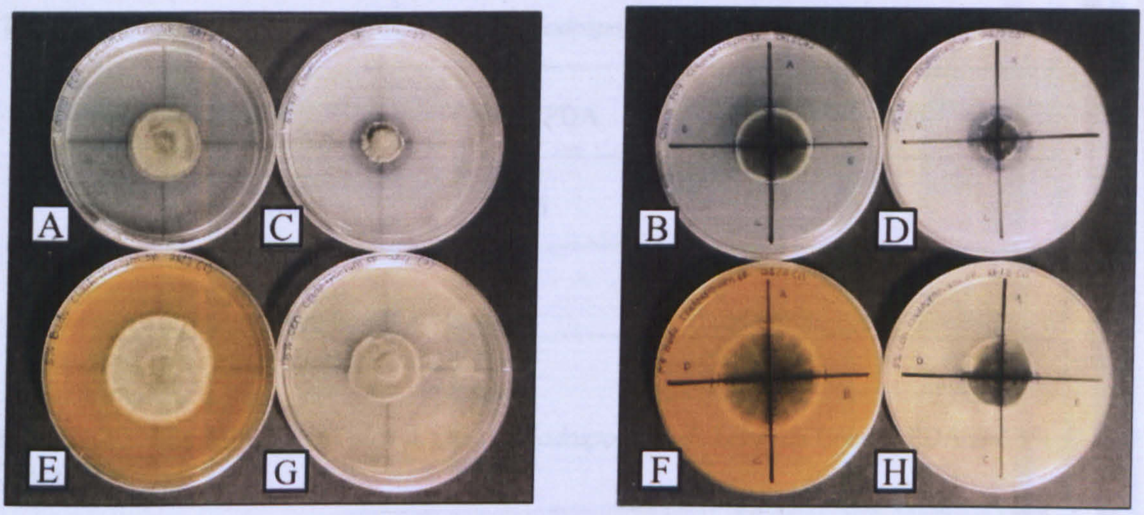

Figure 7. Growth of *Cladosporium sp.* on PDA and 3 different fermented foods mixed with PDA. (a) top plate control PDA, (b) bottom plate control PDA (c) top plate 5% vitagen, (d) bottom plate 5% vitagen, (e) top plate 5% *budu*, (f) bottom plate 5% *budu*, (g) top plate 5% *cencalok*, and (h) bottom plate 5% *cencalok*.

Interestingly, the growth rate of *Cladosporium sp.* on 5% *Budu* showed significantly different compared to growth rate on control PDA, $p<0.05$ while 5% *Cencalok* had no significantly difference compared to growth rate on control PDA, $p>0.05$ respectively (Table 10 and Table 11). Figure 7 above showed the comparison growth of 5% *budu* and 5% *cencalok* compared to PDA. 5% *budu* grow faster than control PDA which revealed weak inhibitory effect of the fermented food (Table 12). These studied were contrast to previous study by Liasi *et al.* (2009). There isolates LAB from *budu* were identified as genus *lactobacillus* (*Lactobacillus casei* LA17, *Lactobacillus plantarum* LA22 and *L. paracasei* LA02). All three isolates show strong inhibitory effects (inhibition zone of 15-18 mm) towards some bacteria such as *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC25923 and *Salmonella enterica* ATCC1331.

Table 10. Comparison mean of growth rate of *Cladosporium sp.* between Control PDA and 5% *Budu.*

	Control PDA	5% *Budu*
Mean Growth	0.16	0.28
P Value	0.29	

Table 11. Comparison mean of growth rate of *Cladosporium sp.* between Control PDA and 5% *Cencalok.*

	Control PDA	5% *Cencalok*
Mean Growth	0.16	0.15
P Value	0.343	

Table 12. Growth rate (cm/day) of *Cladosporium sp.* on control PDA and three different of fermented foods.

Growth rate of *Cladosporium sp.* (cm/day)			
Control PDA	5% Vitagen	5% *Budu*	5% *Cencalok*
0.16	0.04	0.26	0.07
0.16	0.05	0.27	0.14
0.16	0.05	0.28	0.15
0.16	0.05	0.29	0.22

However, there are several limitations while conducting this study. The pH of the medium which included as factor influence of antifungal activity could not be done. Contamination might occur if the measurement of pH level were conducted after autoclaved and mixed of fermented foods in PDA medium. Besides, lactic acid bacteria (LAB) of fermented food especially in local fermented food such as *budu* and *cencalok* could not be identify which LAB cause inhibitory effect because most of study isolates and test LAB from any sources on fungal. Thus, further recommendation would like to suggest in next session.

CHAPTER 5: CONCLUSION & RECOMMENDATION

In conclusion, the inhibition of growth rate of fungi can be observed when tested with culture drinks which are consists of specific strains of bacteria. However, *budu* and *cencalok* show weak inhibition due to their unknown lactic acid bacteria (LAB).Thus, I would like to suggest for further study to isolate and identify bacteria from the fermented food before test for antifungal activity. I also would like to recommend to use overlay assay technique which seem very efficient related to this study.

REFERENCES

Achi, O. K. (2005). The potential for upgrading traditional fermented foods through biotechnology. *African Journal of Biotechnology*, *4*(5), 375-380.

Barkat, E. H.; Du, B.; Ren, Y.; Hardy, G. S. J. & Bayliss, K. L. (2017). Volatile organic compounds associated with postharvest fungi detected in stored wheat grain. *Australasian Plant Pathology*, 46(5), 483-492.

Batish, V. K., Roy, U., Lal, R., & Grower, S. (1997). Antifungal attributes of lactic acid bacteria—a review. *Critical Reviews in Biotechnology*, *17*(3), 209-225.

Bensch, K.; Braun, U.; Groenewald, J. Z. & Crous, P. W. (2012). The genus cladosporium. *Studies in mycology*, *72*, 1-401.

Bulgasem, B. Y., Lani, M. N., Hassan, Z., Wan Yusoff, W. M., & Fnaish, S. G. (2016). Antifungal Activity of Lactic Acid Bacteria Strains Isolated from Natural Honey against Pathogenic Candida Species. *Mycobiology*, *44*(4), 302-309.

Bullerman, L. B. (2003). SPOILAGE | Fungi in Food – An Overview. In Trugo, L. & Finglas, P. M. (Eds.), *Encyclopedia of Food Sciences and Nutrition* (2nd ed). (pp.5511-5522). Lincoln, NE, USA: Academic Press.

Campbell-Platt, G. (1994). Fermented foods—a world perspective. *Food Research International*, *27*(3), 253-257.

Carr, F. J., Chill, D., & Maida, N. (2002). The lactic acid bacteria: a literature survey. *Critical reviews in microbiology*, *28*(4), 281-370.

Coates, L. & Johnson, G. (1997). Postharvest diseases of fruit and vegetables. *Plant Pathogens and Plant Diseases*, 533-548.

Dalié, D. K. D., Deschamps, A. M., & Richard-Forget, F. (2010). Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. *Food control*, *21*(4), 370-380.

De Vuyst, L., & Vandamme, E. J. (1994). Lactic acid bacteria and bacteriocins: their practical importance. In *Bacteriocins of lactic acid bacteria* (pp. 1-11). Springer, Boston, MA.

Dhoro, M. (2012). Identification and differentiation of Fusarium species using selected molecular methods. (Master's thesis, University of Zimbabwe).

Hassan, Y. I., & Bullerman, L. B. (2008). Antifungal activity of *Lactobacillus paracasei ssp.* tolerans isolated from a sourdough bread culture. *International Journal of Food Microbiology*, *121*(1), 112-115.

Hajeb, P., & Jinap, S. (2012). Fermented shrimp products as source of umami in Southeast Asia. *J Nutr Food Sci S, 10*, 006.

Huda, N. (2012). Indonesian fermented fish products. *Handbook of Animal-Based Fermented Food and Beverage Technology, 2*, 717-738.

Hussin, N.A.; Bolhassan, M. H. & San, Y. F. K. (2016). Antifungal properties of elephantopus scaber L. (Asteraceae) against crop pathogenic fungi. *Borneo Journal of Resource Science and Technology, 6*(2), 48-52.

Irianto, H. E. (2017). Enzymes in Fermented Fish. In *Advances in food and nutrition research* (Vol. 80, pp. 199-216). Academic Press.

Kroger, M., Kurmann, J. A., & Rasic, J. L. (1992). Fermented Milks—Past, Present. *Applications of Biotechnology to Traditional Fermented Foods: Report of an Ad Hoc Panel of the Board on Science and Technology for International Development*, 61.

Lindgren, S., & Pleje, M. (1983). Silage fermentation of fish or fish waste products with lactic acid bacteria. *Journal of the Science of Food and Agriculture, 34*(10), 1057-1067.

Magnusson, J., Ström, K., Roos, S., Sjögren, J., & Schnürer, J. (2003). Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS microbiology letters, 219*(1), 129-135.

Man, N., Nawi, N. M. & Ismail, M. M. (2009). An overview of the supply chain management of malaysian vegetable and fruit industries focusing on the channel of distribution. *Journal of Agribusiness Marketing,* 2, 1-18.

Nelson, P. E. (1992). Taxonomy and biology of *Fusarium moniliforme*. *Mycopathologia, 117*(1), 29-36.

Oberman, H. & Libudzisz, Z. (1998). Fermented milks. In *Microbiology of fermented foods* (pp. 308-350). Springer, Boston, MA.

Olonisakin, O. O., Jeff-Agboola, Y. A., Ogidi, C. O., & Akinyele, B. J. (2017). Isolation of Antifungal Lactic Acid Bacteria (LAB) from "Kunu" against Toxigenic *Aspergillus flavus*. *Preventive nutrition and food science, 22*(2), 138.

Sathe, S. J., Nawani, N. N., Dhakephalkar, P. K., & Kapadnis, B. P. (2007). Antifungal lactic acid bacteria with potential to prolong shelf-life of fresh vegetables. *Journal of Applied Microbiology, 103*(6), 2622-2628.

Schnürer, J., & Magnusson, J. (2005). Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science & Technology, 16*(1-3), 70-78.

Shiby, V. K., & Mishra, H. N. (2013). Fermented milks and milk products as functional foods—A review. *Critical reviews in food science and nutrition, 53*(5), 482-496.

Sim, K. Y., Chye, F. Y., & Anton, A. (2015). Chemical composition and microbial dynamics of budu fermentation, a traditional Malaysian fish sauce. *Acta Alimentaria, 44*(2), 185-194.

Soomro, A. H., Masud, T., & Anwaar, K. (2002). Role of lactic acid bacteria (LAB) in food preservation and human health-a review. *Pakistan Journal of Nutrition, 1*(1), 20-24.

Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. *International journal of food microbiology, 36*(1), 1-29.

Summerell, B. A., Salleh, B. & Leslie, J.F. (2003). A utilitarian approach to Fusarium identification. *Plant disease, 87*(2), 117-128.

Tropcheva, R., Nikolova, D., Evstatieva, Y., & Danova, S. (2014). Antifungal activity and identification of *Lactobacilli*, isolated from traditional dairy product "katak". *Anaerobe, 28*, 78-84.

Tournas, V. H. (2005). Spoilage of vegetable crops by bacteria and fungi and related health hazards. *Critical reviews in microbiology, 31*(1), 33-44.

Vitagen.com. (2017). Product: VITAGEN Regular. Retrieved on 2018, 29 April from www.vitagen.com.my/products/regular.html

Walker, C.; Muniz, M. F. B.; Rolim, J. M.; Martins, R. R. O.; Rosenthal, V. C.; Maciel, C. G.; Mezzomo, R. & Reiniger, L. R. S. (2016). Morphological and molecular characterization of *Cladosporium cladosporioides* species complex causing pecan tree leaf spot. *Genetic Molecular Research, 16*(15), 3.

Wilson, C. L.; Wisniewski, M. E.; Biles, C. L.; McLaughlin, R.; Chalutz, E. & Droby, S. (1991). Biological control of post-harvest diseases of fruits and vegetables: alternatives to synthetic fungicides. *Crop protection, 10*(3), 172-177.

Yakult.co.in. Yakult history. Retrieved on 2018, 29 April from https://www.yakult.co.in/yakult-history.php

APPENDICES

Appendix 1. Raw calculation of *Fusarium* white (Control)

Control 1							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.6	1.1	1.4	1.9	2.3	2.6	3.15
B	0.6	1.1	1.45	1.9	2.3	2.7	3.1
C	0.65	1.05	1.5	1.85	2.3	2.65	3.1
D	0.7	1.15	1.55	1.95	2.35	2.7	3.15
Colony growth	0.6375	1.1	1.475	1.9	2.3125	2.6625	3.125
	0.4625	0.375	0.425	0.4125	0.35	0.4625	
Growth rate	0.414583						

Control 2							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.7	1.1	1.5	1.95	2.4	2.7	3.2
B	0.6	1	1.5	1.85	2.25	2.65	3
C	0.6	1.1	1.5	1.9	2.35	2.65	3.1
D	0.1	1.1	0.58	0.95	2.45	2.7	3.15
Colony growth	0.5	1.075	1.27	1.6625	2.3625	2.675	3.1125
	0.575	0.195	0.3925	0.7	0.3125	0.4375	
Growth rate	0.435417						

Control 3							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.65	1.05	1.5	1.85		2.55	3
B	0.65	1.05	1.45	1.9	2.25	2.65	3.05
C	0.65	1.05	1.45	1.9	2.3	2.7	3.1
D	0.7	1.1	1.5	1.9	2.3	2.65	3
Colony growth	0.675	1.075	1.475	1.9	2.275	2.65	3.025
	0.4	0.4	0.425	0.375	0.375	0.375	
Growth rate	0.391667						

Control 4							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.6	1.1	1.5	1.9	2.25	2.65	3.05
B	0.65	1.1	1.5	1.9	2.3	2.7	3.1
C	0.65	1.1	1.5	1.9	2.3	2.65	3.15
D	0.6	1.05	1.5	1.9	2.25	2.65	3
Colony growth	0.625	1.0875	1.5	1.9	2.275	2.6625	3.075
	0.4625	0.4125	0.4	0.375	0.3875	0.4125	
Growth rate	0.408333						

Appendix 2. Raw calculation of *Fusarium* white (5% Vitagen)

5% VIT 1							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.65	1.05	1.45	1.8	2.15	2.45	2.75
B	0.55	1.05	1.45	1.8	2.15	2.45	2.75
C	0.7	1.1	1.55	1.85	2.3	2.5	2.85
D	0.6	1.05	1.45	1.8	2.2	2.45	2.75
Colony growth	0.625	1.0625	1.475	1.8125	2.2	2.4625	2.775
	0.4375	0.4125	0.3375	0.3875	0.2625	0.3125	
Growth rate	0.358333						

5% VIT 2							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.65	0.95	1.45	1.8	2.15	2.45	2.75
B	0.65	1	1.45	1.8	2.2	2.45	2.75
C	0.85	1.15	1.6	1.95	2.3	2.6	2.9
D	0.7	1.05	1.5	2.25	2.5	2.8	3.1
Colony growth	0.7125	1.0375	1.5	1.95	2.2875	2.575	2.875
	0.325	0.4625	0.45	0.3375	0.2875	0.3	
Growth rate	0.360417						

5% VIT 3							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.7	1.1	1.45	1.85	2.15	2.45	2.75
B	0.7	1.05	1.45	1.75	2.15	2.4	2.7
C	0.6	1.05	1.45	1.75	2.15	2.4	2.7
D	0.7	1.1	1.5	1.85	2.2	2.45	2.75
Colony growth	0.675	1.075	1.4625	1.8	2.1625	2.425	2.725
	0.4	0.3875	0.3375	0.3625	0.2625	0.3	
Growth rate	0.341667						

5% VIT 4							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.7	1.1	1.5	1.85	2.1	2.45	2.8
B	0.65	1	1.45	1.8	2.1	2.45	2.75
C	0.65	1.05	1.45	1.8	2.15	2.45	2.8
D	0.7	1.1	1.55	1.9	2.25	2.55	2.85
Colony growth	0.675	1.0625	1.4875	1.8375	2.15	2.475	2.8
	0.3875	0.425	0.35	0.3125	0.325	0.325	
Growth rate	0.354167						

Appendix 3. Raw calculation of *Fusarium* white (5% Budu)

5% BUDU 1							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.6	1	1.35	1.85	2.3	2.7	3.2
B	0.65	1.05	0.45	1.85	2.45	2.8	3.25
C	0.7	1.1	1.6	1.95	2.4	2.8	3.3
D	0.7	1.1	1.6	1.95	2.4	2.85	3.3
Colony growth	0.6625	1.0625	1.25	1.9	2.3875	2.7875	3.2625
	0.4	0.1875	0.65	0.4875	0.4	0.475	
Growth rate	0.433333						

5% BUDU 2							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.6	1.05	1.5	1.9	2.4	2.75	3.3
B	0.7	1.05	1.55	1.95	2.35	2.8	3.3
C	0.65	1.05	1.55	1.95	2.4	2.85	3.3
D	0.65	1.05	1.5	1.9	2.3	2.8	3.25
Colony growth	0.65	1.05	1.525	1.925	2.3625	2.8	3.2875
	0.4	0.475	0.4	0.4375	0.4375	0.4875	
Growth rate	0.439583						

5% BUDU 3							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.6	1.05	1.5	1.9	2.35	2.75	3.2
B	0.65	1.05	1.5	1.95	2.35	2.75	3.2
C	0.7	1.1	1.6	2	2.4	2.85	3.3
D	0.65	1.1	1.55	1.95	2.35	2.8	3.3
Colony growth	0.65	1.075	1.5375	1.95	2.3625	2.7875	3.25
	0.425	0.4625	0.4125	0.4125	0.425	0.4625	
Growth rate	0.433333						

5% BUDU 4							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.7	1.1	1.55	2	2.45	2.85	3.3
B	0.6	1.05	1.5	1.9	2.45	2.8	3.25
C	0.6	1.05	1.5	1.9	2.4	2.8	3.25
D	0.7	1.15	1.6	2	2.45	2.9	3.3
Colony growth	0.65	1.0875	1.5375	1.95	2.4375	2.8375	3.275
	0.4375	0.45	0.4125	0.4875	0.4	0.4375	
Growth rate	0.4375						

Appendix 4. Raw calculation of *Fusarium* white (5% Cencalok)

5% CEN 1							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.7	1.2	1.7	2.05	2.5	2.85	3.35
B	0.65	1.2	1.65	1.95	2.4	2.85	3.2
C	0.6	1.2	1.6	2	2.4	2.85	3.2
D	0.7	1.25	1.7	2.1	2.45	2.8	3.35
Colony growth	0.6625	1.2125	1.6625	2.025	2.4375	2.8375	3.275
	0.55	0.45	0.3625	0.4125	0.4	0.4375	
Growth rate	0.435417						

5% CEN 2							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.75	1.25	1.85	2.25	2.8	3.15	3.7
B	0.65	1.25	1.75		2.7	3.15	3.7
C	0.65	1.15	1.75	2.2	2.75	3.2	3.75
D	0.7	1.25	1.8	2.25	2.85	3.4	4
Colony growth	0.7	1.2	1.8	2.225	2.775	3.175	3.725
	0.5	0.6	0.425	0.55	0.4	0.55	
Growth rate	0.504167						

5% CEN 3							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.7	1.25	1.7	2.1	2.5	2.85	3.25
B	0.8	1.1	1.6	2	2.5	2.85	3.3
C	0.65	1.2	1.7	2.05			3.25
D	0.75	1.25	1.75	2.1	2.5	2.85	3.5
Colony growth	0.775	1.175	1.675	2.05	2.5	2.85	3.4
	0.4	0.5	0.375	0.45	0.35	0.55	
Growth rate	0.4375						

5% CEN 4							
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
A	0.65	1.2	2.15	2.6	3	3.55	4
B	0.65	1.15	1.65	2.1	2.5	3.3	4
C	0.7	1.25	1.8	2.2	2.65	3.15	3.6
D	0.8	1.4	1.85	2.25	2.7	3.18	3.7
Colony growth	0.7	1.25	1.8625	2.2875	2.7125	3.295	3.825
	0.55	0.6125	0.425	0.425	0.5825	0.53	
Growth rate	0.520833						

Appendix 5. Raw calculation of *Fusarium* pink (Control)

Control 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY8
A	0.5	0.85	1.1	1.45	1.75	2	2.25	2.5
B	0.45	0.75	1	1.3	1.55	1.85	2.1	2.35
C	0.5	0.75	1	1.25	1.5	1.75	2	2.2
D	0.5	0.75	1	1.35	1.6	1.85	2.1	2.35
Colony growth	0.4875	0.775	1.025	1.3375	1.6	1.8625	2.1125	2.35
	0.2875	0.25	0.3125	0.2625	0.2625	0.25	0.2375	
Growth rate	0.26607							

Control 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY8
A	0.55	0.9	1.2	1.55	1.8	2.15	2.4	2.6
B	0.45	0.7	1.05	1.45	1.65	1.95	2.2	2.45
C	0.5	0.75	1.05	1.4	1.6	1.95	2.2	2.4
D	0.55	0.85	1.1	1.4	1.6	2	2.25	2.4
Colony growth	0.5125	0.8	1.1	1.45	1.6625	2.0125	2.2625	2.4625
	0.2875	0.3	0.35	0.2125	0.35	0.25	0.2	
Growth rate	0.27857							

Control 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY8
A	0.5	0.8	1.15	1.45	1.7	1.95	2.3	2.5
B	0.4	0.7	1	1.3	1.55	1.8	2	2.25
C	0.5	0.75	1	1.3	1.5	1.8	2.1	2.3
D	0.5	0.75	1.1	1.4	1.7	1.95	2.3	2.45
Colony growth	0.475	0.75	1.0625	1.3625	1.6125	1.875	2.175	2.375
	0.275	0.3125	0.3	0.25	0.2625	0.3	0.2	
Growth rate	0.27143							

Control 4								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.5	0.8	1.15	1.45	1.7	1.95	2.3	2.5
B	0.4	0.7	1	1.3	1.55	1.8	2	2.25
C	0.5	0.75	1.1	1.4	1.7	1.95	2.1	2.3
D	0.5	0.75	1.1	1.4	1.7	1.95	2.3	2.45
Colony growth	0.475	0.75	1.0875	1.3875	1.6625	1.9125	2.175	2.375
	0.275	0.3375	0.3	0.275	0.25	0.2625	0.2	
Growth rate	0.27143							

Appendix 6. Raw calculation of *Fusarium* pink (5% Vitagen)

5% VIT 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.45	0.7	0.9	1.05	1.15	1.35	1.55	1.75
B	0.55	0.75	0.95	1.15	1.25	1.45	1.65	1.8
C	0.55	0.8	1.0	1.015	1.25	1.45	1.6	1.8
D	0.45	0.7	0.95	1.05	1.15	1.35	1.55	1.75
Colony growth	0.5	0.7375	0.95	1.0665	1.2	1.4	1.5875	1.775
	0.2375	0.2125	0.11625	0.13375	0.2	0.1875	0.1875	
Growth rate	0.18214							

5% VIT 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY8
A	0.5	0.75	0.9	1.05	1.15	1.35	1.5	1.7
B	0.5	0.75	0.95	1.1	1.2	1.4	1.55	1.7
C	0.5	0.65	0.9	1.05	1.15	1.3	1.5	1.65
D	0.45	0.6	0.8	0.95	1.05	1.25	1.45	1.6
Colony growth	0.4875	0.6875	0.8875	1.0375	1.1375	1.325	1.5	1.6625
	0.2	0.2	0.15	0.1	0.1875	0.175	0.1625	
Growth rate	0.167857							

5% VIT 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY8
A	0.55	0.75	0.95	1.1	1.2	1.35	1.6	1.75
B	0.45	0.65	0.9	1.05	1.15	1.3	1.55	1.7
C	0.45	0.65	0.85	1.05	0.15	1.3	1.5	1.65
D	0.5	0.7	0.95	1.1	1.25	1.4	1.55	1.75
Colony growth	0.4875	0.6875	0.9125	1.075	0.9375	1.3375	1.55	1.7125
	0.2	0.225	0.1625	-0.1375	0.4	0.2125	0.1625	
Growth rate	0.175							

5% VIT 4								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY8
A	0.5	0.7	0.95	1.1	1.2	1.4	1.6	1.75
B	0.45	0.7	0.9	1.1	1.2	1.35	1.55	1.7
C	0.5	0.75	0.9	1.05	1.2	1.4	1.55	1.75
D	0.5	0.7	0.95	1.1	1.2	1.4	1.55	1.75
Colony growth	0.4875	0.7125	0.925	1.0875	1.2	1.3875	1.5625	1.7375
	0.225	0.2125	0.1625	0.1125	0.1875	0.175	0.175	
Growth rate	0.178571							

Appendix 7. Raw calculation of *Fusarium* pink (5% Budu)

5% BUDU 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY5	DAY 6	DAY 7	DAY 8
A	0.55	0.8	1.3	1.7	2.25	2.65	3.05	3.4
B	0.5	0.8	1.3	1.7	2.3	2.65	3	3.25
C	0.55	0.85	1.3	1.8	2.4	2.8	3.25	3.5
D	0.55	0.85	1.3	1.75	2.4			
Colony growth	0.55	0.825	1.3	1.75	2.325	2.725	3.15	3.45
	0.275	0.475	0.45	0.575	0.4	0.425	0.3	
Growth rate	0.414286							

5% BUDU 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY5	DAY 6	DAY 7	DAY 8
A	0.55	0.85	1.4	1.8	2.1	2.4	2.7	3
B	0.6	0.95	1.45	1.85	2.2	2.5	2.8	3.1
C	0.5	0.9	1.4	1.85	2.15	2.45	2.8	3.15
D	0.5	0.85	1.35	1.8	2.1	2.4	2.7	3
Colony growth	0.5375	0.8875	1.4	1.825	2.1375	2.4375	2.75	3.0625
	0.35	0.5125	0.425	0.3125	0.3	0.3125	0.3125	
Growth rate	0.360714							

5% BUDU 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY5	DAY 6	DAY 7	DAY 8
A	0.5	0.85	1.3	1.65	2	2.4		
B	0.45	0.85	1.3	1.7	2	2.4	2.75	3.1
C	0.5	0.9	1.35	1.8	2.15	2.5	2.95	3.25
D	0.55	0.9	1.35	1.8	2.15	2.5	2.95	3.25
Colony growth	0.5	0.875	1.325	1.75	2.075	2.45	2.85	3.175
	0.375	0.45	0.425	0.325	0.375	0.4	0.325	
Growth rate	0.382143							

5%BUDU 4								
	DAY 1	DAY2	DAY 3	DAY 4	DAY5	DAY 6	DAY 7	DAY 8
A	0.55	0.9	1.35	1.8	2.05	2.35	2.6	2.95
B	0.55	0.95	1.3	1.75	2.05	2.3	2.6	2.95
C	0.5	0.85	1.35	1.7	2	2.25	2.6	2.9
D	0.5	0.9	1.35	1.7	2.05	2.3	2.6	2.95
Colony growth	0.525	0.9	1.3375	1.7375	2.0375	2.3	2.6	2.9375
	0.375	0.4375	0.4	0.3	0.2625	0.3	0.3375	
Growth rate	0.344643							

Appendix 8. Raw calculation of *Fusarium* pink (5% Cencalok)

5% CEN 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.7	1.1	1.5	1.85	2.05	2.25	2.4	2.7
B	0.6	1	1.4	1.75	2	2.2	2.45	2.7
C	0.55	0.95	1.35	1.7	1.95	2.2	2.45	2.7
D	0.65	0.95	1.45	1.75	1.9	2.15	2.35	2.5
Colony growth	0.625	1	1.425	1.7625	1.975	2.2	2.4125	2.65
	0.375	0.425	0.3375	0.2125	0.225	0.2125	0.2375	
Growth rate	0.289286							

5% CEN 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.6	0.95	1.45	1.75	1.95	2.15	2.35	2.6
B	0.65	0.95	1.4	1.75	2	2.15	2.4	2.6
C	0.65	0.95	1.4	1.8	2.1	2.25	2.45	2.6
D	0.65	1	1.5	1.85	2.05	2.2	2.45	2.7
Colony growth	0.6375	0.9625	1.4375	1.7875	2.025	2.1875	2.4125	2.625
	0.325	0.475	0.35	0.2375	0.1625	0.225	0.2125	
Growth rate	0.283929							

5% CEN 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.6	1.05	1.5	1.9	2.15	2.3	2.55	2.75
B	0.65	1.1	1.65	2	2.2	2.4	2.6	2.8
C	0.65	0.95	1.45	1.85	2.05	2.25	2.4	2.55
D	0.6	0.95	1.45	1.85	2.05	2.2	2.45	2.6
Colony growth	0.625	1.0125	1.5125	1.9	2.1125	2.2875	2.5	2.675
	0.3875	0.5	0.3875	0.2125	0.175	0.2125	0.175	
Growth rate	0.292857							

5% CEN 4								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.6	0.95	1.5	1.95	2.35	2.7	3.05	3.2
B	0.65	0.95	1.4	1.9	2.15	2.35	2.55	2.7
C	0.6	1	1.45	1.9	2.15	2.35		2.7
D	0.6	0.95	1.45	1.9	2.2	2.45	2.6	2.75
Colony growth	0.625	0.95	1.425	1.9	2.175	2.4	2.575	2.725
	0.325	0.475	0.475	0.275	0.225	0.175	0.15	
Growth rate	0.30							

Appendix 9. Raw calculation of *Cladosporium sp.* (Control)

control 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.5	0.75	0.9	1.05	1.25	1.4	1.5	1.7
B	0.5	0.75	0.95	1.1	1.25	1.35	1.55	1.65
C	0.45	0.65	0.8	1	1.1	1.25		1.55
D	0.45	0.65	0.8	1	1.1	1.25	1.35	1.5
Colony growth	0.475	0.7	0.875	1.05	1.175	1.3	1.45	1.575
	0.225	0.175	0.175	0.125	0.125	0.15	0.125	
Growth rate	0.15714							

control 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY 8
A	0.45	0.7						
B	0.45	0.75	0.9	1.1	1.15	1.3	1.5	1.6
C	0.5	0.075	0.95	1.1	1.2	1.35	1.55	1.65
D	0.5	0.75	0.95	1.1	0.2	1.35	1.55	1.65
Colony growth	0.475	0.75	0.925	1.1	0.675	1.325	1.525	1.625
	0.275	0.175	0.175	0.425	0.65	0.2	0.1	
Growth rate	0.16429							

control 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.55	0.75	0.9	1.1	1.2	1.35	1.5	1.6
B	0.45	0.7	0.9	1.05	1.2	1.3	1.45	1.6
C	0.45	0.65	0.85	1				
D	0.5	0.7	0.9	1.05	1.2	1.3	1.5	1.6
Colony growth	0.475	0.7	0.9	1.05	1.2	1.3	1.475	1.6
	0.225	0.2	0.15	0.15	0.1	0.175	0.125	
Growth rate	0.16071							

control 4								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY 8
A	0.45	0.65	0.8	1.05	1.15	1.3	1.5	1.6
B	0.55	0.75	0.95	1.2	1.35	1.5	1.65	1.8
C	0.5	0.7	0.9	1.85	1.2	1.5	1.45	1.6
D	0.5	0.65	0.8	0.95	1.15	1.25	1.4	1.5
Colony growth	0.5	0.6875	0.8625	1.2625	1.2125	1.3875	1.5	1.625
	0.1875	0.175	0.4	-0.05	0.175	0.1125	0.125	
Growth rate	0.16071							

Appendix 10. Raw calculation of *Cladosporium sp.* (5% Vitagen)

5% VIT 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY8
A	0.5	0.6	0.6	0.7	0.7	0.8	0.8	0.85
B	0.5	0.6	0.6	0.7	0.7	0.8	0.8	0.85
C	0.55	0.65	0.65	0.75	0.75	0.85	0.85	0.9
D	0.55	0.6	0.6	0.7	0.7	0.8	0.8	0.85
Colony growth	0.525	0.6125	0.6125	0.7125	0.7125	0.8125	0.8125	0.8625
	0.0875	0	0.1	0	0.1	0	0.05	
Growth rate	0.04821							

5% VIT 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY8
A	0.55	0.65	0.65	0.75	0.75	0.8	0.8	0.85
B	0.55	0.65	0.65	0.75	0.75	0.85	0.85	0.9
C	0.45	0.55	0.55	0.65	0.65	0.7	0.7	0.75
D	0.45	0.5	0.5	0.55	0.55	0.65	0.65	0.75
Colony growth	0.5	0.5875	0.5875	0.675	0.675	0.75	0.75	0.8125
	0.0875	0	0.0875	0	0.075	0	0.0625	
Growth rate	0.04464							

5% VIT 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY8
A	0.55	0.7	0.7	0.8	0.8	0.85	0.85	0.9
B	0.55	0.7	0.7	0.8	0.8	0.9	0.9	0.95
C	0.5	0.6	0.6	0.7	0.7	0.8	0.8	0.85
D	0.4	0.5	0.5	0.6	0.6	0.7	0.7	0.75
Colony growth	0.5	0.625	0.625	0.725	0.725	0.8125	0.8125	0.8625
	0.125	0	0.1	0	0.0875	0	0.05	
Growth rate	0.05179							

5% VIT 4								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY8
A	0.45	0.5	0.5	0.6	0.6	0.7	0.7	0.75
B	0.45	0.55	0.55	0.65	0.65	0.7	0.7	0.8
C	0.55	0.65	0.65	0.75	0.75	0.85	0.85	0.9
D	0.55	0.65	0.65	0.7	0.75	0.85	0.85	
Colony growth	0.5	0.575	0.575	0.675	0.675	0.775	0.775	0.825
	0.075	0	0.1	0	0.1	0	0.05	
Growth rate	0.046429							

Appendix 11. Raw calculation of *Cladosporium sp.* (5% Budu)

5% BUDU 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY 8
A	0.55	0.8	1.05	1.35	1.65	1.9	2.15	2.4
B	0.45	0.8	1.1	1.35	1.6	1.9	2.1	2.4
C	0.55	0.8	1.1	1.4	1.6	1.9	2.15	2.4
D	0.55	0.8	1.15	1.35	1.65	1.95	2.2	2.4
Colony growth	0.525	0.8	1.1	1.3625	1.625	1.9125	2.15	2.4
	0.275	0.3	0.2625	0.2625	0.2875	0.2375	0.25	
Growth rate	0.26786							

5% BUDU 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY 8
A	0.55	0.8	1.1	1.3	1.6	1.85	2.05	2.35
B	0.5	0.7	1	1.25	1.5	1.8	2.05	2.25
C	0.55	0.85	1.1	1.4	1.6	1.95	2.15	2.35
D	0.5	0.75	1.05	1.5	1.85	2.05	2.3	2.45
Colony growth	0.525	0.775	1.0625	1.3625	1.6375	1.9125	2.1375	2.35
	0.25	0.2875	0.3	0.275	0.275	0.225	0.2125	
Growth rate	0.26071							

5% BUDU 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY 8
A	0.6	0.9	1.2	1.5	1.8	2.05	2.35	2.6
B	0.5	0.85	1.1	1.4	1.7		2.35	2.5
C	0.45	0.75	1.05	1.35	1.65	1.95	2.25	2.5
D	0.6	0.85	1.15	1.45	1.7	2	2.3	2.6
Colony growth	0.525	0.825	1.125	1.425	1.725	2	2.3	2.55
	0.3	0.3	0.3	0.3	0.275	0.3	0.25	
Growth rate	0.28929							

5% BUDU 4								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY 8
A	0.5	0.8	1.05	1.3	1.55	1.95	2.15	2.45
B	0.5	0.8	1.05	1.35	1.55	1.95	2.2	2.45
C	0.5	0.8	1.05	1.4	1.65	2.05	2.2	2.45
D	0.5	0.8	1.05	1.4	1.65	2	2.2	2.45
Colony growth	0.5	0.8	1.05	1.3625	1.6	1.9875	2.1875	2.45
	0.3	0.25	0.3125	0.2375	0.3875	0.2	0.2625	
Growth rate	0.27857							

Appendix 12. Raw calculation of *Cladosporium sp.* (5% Cencalok)

5% CEN 1								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.55	0.9	1.05	1.2	1.3	1.45	1.6	1.7
B	0.45	0.8	0.95	1.1	1.15	1.3	1.3	1.4
C	0.45	0.75	0.9	1.05	1.15	1.25		
D	0.55	0.85	1	1.15	1.25	1.4	1.55	1.6
Colony growth	0.5	0.825	0.975	1.125	1.2	1.35	1.425	1.5
	0.325	0.15	0.15	0.075	0.15	0.075	0.075	
Growth rate	0.14286							

5% CEN 2								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.45	0.75	0.85	0.85	0.85	0.95	0.95	0.95
B	0.5	0.9	1	1	1	1.1	1.1	1.1
C	0.6	0.9	1	1	1	1.1	1.1	1.1
D	0.55	0.85	0.95	0.95	0.95	1	1	1
Colony growth	0.525	0.85	0.95	0.95	0.95	1.0375	1.0375	1.0375
	0.325	0.1	0	0	0.0875	0	0	
Growth rate	0.07321							

5% CEN 3								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY6	DAY 7	DAY 8
A	0.5	0.8	0.95	1.2				
B	0.55	0.85	1.05	1.25	1.35	1.5	1.55	1.6
C	0.55	0.85	1	1.15	1.3	1.35	1.5	
D	0.55	0.8	0.95	1.1	1.25	1.3	1.4	1.55
Colony growth	0.55	0.825	1	1.175	1.3	1.4	1.475	1.575
	0.275	0.175	0.175	0.125	0.1	0.075	0.1	
Growth rate	0.14643							

5% CEN 4								
	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8
A	0.55	0.85	1	1.2	-	1.65	1.9	2.1
B	0.6	0.9	1	1.2	1.4	1.7	1.9	2.15
C	0.55	0.8	0.95	1.1	1.2	1.3	1.5	
D	0.45	0.75	0.9	1.1	1.3	1.5	1.75	1.95
Colony growth	0.525	0.825	0.95	1.15	1.35	1.6	1.825	2.05
	0.3	0.125	0.2	0.2	0.25	0.225	0.225	
Growth rate	0.21786							